2015 IRIC NEXT GENERATION AWARDS

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INSTITUTE FOR RESEARCH IN IMMUNOLOGY AND CANCER

Université de Montréal
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Internship project # 1

**Molecular controls of cell division**
Under the supervision of Vincent Archambault
IRIC - Cell Cycle Regulation Research Unit

**PROJECT DESCRIPTION**

Ongoing projects aim at understanding in details the molecular mechanisms that orchestrate the events of the cell division cycle. Our efforts concentrate on the roles of kinase and phosphatase enzymes that are perturbed in cancers. The selected student will work as part of a team in a multidisciplinary project that will maximise his/her experience in the lab.

**LAB TECHNIQUES**

- Fluorescence microscopy
- Biochemistry
- Molecular biology
- *Drosophila melanogaster*
- Cell culture

**FOR MORE INFO**

[www.archambault.iric.ca/research.html](http://www.archambault.iric.ca/research.html)
PROJECT DESCRIPTION

Based on the 3D crystal structure of the β2-adrenergic receptor, the Bouvier laboratory has identified receptor domains and residues that play specific roles in activating distinct downstream effectors (ex: Gs, Gi, β-arrestins, cAMP production, MAPK activation, endocytosis, etc). This allows proposing a molecular/structural basis explaining ligand-biased signalling (i.e. different ligands can modulate distinct downstream effectors with different efficacies) of G protein-coupled receptors. The project will aim at assessing the influence of the diverse receptor domains and residues in the functionally selective response of a collection of β-adrenergic ligands. The response profiles obtained for different ligands will then be translated in structural terms to determine the receptor conformational states responsible for ligand-biased functional selectivity. This project should provide new pharmacological and structural knowledge that could be used to rationally design new classes of drugs with define signalling selectivity and thus, improved therapeutic potential.

LAB TECHNIQUES

Site-directed mutagenesis
Cell culture
Protein heterologous expression
Western blot analysis
ELISA
Receptor activity and second messenger generation using BRET-based biosensors

FOR MORE INFO

www.iri.ca/en/research/principal-investigators/michel-bouvier
PROJECT DESCRIPTION

Mutations in the gene encoding the melanocortin type-4 receptor (MC4R) represent the largest monogenetic cause of early onset severe obesity; a disease increasing the probability of developing cardiopulmonary diseases as well as divers cancers. The Bouvier laboratory discovered that the majority of the disease-causing MC4R mutations (more than 75) lead to the intracellular retention of the receptor due to improper folding and recognition by the endoplasmic reticulum quality control system of the cells. This miss-targeting of the receptor is the cause for the loss of function phenotype and the development of the obesity phenotype resulting from the loss of appetite regulation and energy expenditure under the control of MC4R. The Bouvier laboratory has also identified small drug-like molecules, known as pharmacological chaperones, that can rescue the cell-surface targeting of the receptor and restores the signalling activity of at least one of the signalling cascades (Gs-mediated activation of the adenylyl cyclase and cAMP production) engaged by the MC4R. These pharmacological chaperones therefore represent drug candidates for the development of novel therapies for MC4R-caused obesity. However, the MC4R can also engage other signalling pathways. These include the activation of MAP-kinases, recruitment of β-arrestin and most likely the stimulation of additional G proteins. The present project therefore aims at determining if the MC4R pharmacological chaperones can restore the ability of the mutant forms of the receptor toward these different pathways. For this purpose one of the most frequent mutation R165WMC4R will be used as a test case. We also have generated a mouse model harbouring this obesity-causing mutation, allowing to test the efficacy of the pharmacological chaperones in vivo.

LAB TECHNIQUES

Site-directed mutagenesis
Cell culture
Protein heterologous expression
Western blot analysis
ELISA
Receptor activity and second messenger generation using BRET-based biosensors

FOR MORE INFO

www.irc.ca/en/research/principal-investigators/michel-bouvier
PROJECT DESCRIPTION

90% of cancer patients die from abnormal migration of cancer cells (metastasis) throughout the body. The epithelial-mesenchymal transition (EMT) allows cells to acquire the ability to migrate through the body. This process is normally restricted to the development of the embryo and is therefore turned off after birth. However, cancer cells are able to reprogram the TEM to migrate and to form metastases. Understanding how cancer cells reprogram the TEM is therefore an important challenge for fundamental and biomedical research. Our laboratory has discovered a mechanism that blocks the TEM in healthy cells (JCB 2008 JCB 2011 JCB 2013). Our work suggests that cancer cells bypass this mechanism to reprogram the TEM and metastasize. This project aims to better understand the basic mechanism that we have identified and thus better understand how cancer cells are reprogramed. It is of crucial importance since it will allow to define targeted anti-metastatic strategies to fight against cancer.

LAB TECHNIQUES

Molecular biology
Cell biology
Biochemistry
5D time-lapse microscopy

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/sebastien-carreno
PROJECT DESCRIPTION

The overall goal of this project is to study the role of the cell cycle machinery in cellular proliferation. Defects in the cell cycle machinery often cause diseases associated with increased susceptibility to cancer in humans. The main objective of the internship is to identify the natural substrates of the CDK and PLK kinases, and to study how the loss of substrate phosphorylation impacts cell proliferation and chromosome stability. This research project touches on several important aspects of cell biology, such as the regulation of chromosome structure and its connection to signal transduction pathways. Throughout this internship, the trainee will be using model organisms to learn advanced techniques in proteomics, microscopy and genetics.

LAB TECHNIQUES

Cell biology (wild-type and mutant cell observation by fluorescence microscopy)
Genetics (gene deletion and point-mutant construction)
Molecular biology (cloning, PCR, mutagenesis)
Proteomics (phosphorylation site identification in proteins)

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/damien-damours
PROJECT DESCRIPTION

Collective cell migration is involved in many developmental events, but also in pathologies and in the dissemination of cancer cells in particular. By using the Drosophila model system, we have identified new regulators of collective cell migration among which the small GTPase Ral. The student will further analyse the mechanisms disrupted when Ral is downregulated in border cells, which migrate as small cluster in the the Drosophila egg chambers.

LAB TECHNIQUES

Drosophila genetics
Confocal microscopy
General cell biology techniques

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/gregory-emery
www.emery.iric.ca
PROJECT DESCRIPTION

Acute leukemias are maintained by a rare subpopulation of leukemic stem cells that can escape chemotherapy. We have identified novel small molecule inhibitors of leukemic stem cells. The project consists in analysing how validated hits kill leukemic stem cells.

LAB TECHNIQUES

Transgenic mouse models of acute leukemia
Flow cytometry
Cell purification
RT-PCR
Cell culture
Transplantation assays

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/trang-hoang
PROJECT DESCRIPTION

The mitotic kinesin kif14 has been shown to be overexpressed in multiple cancers. Its overexpression correlates positively with disease progression and poor prognosis. In addition, kif14 is a prime candidate oncogene on chromosome 1q32, a hot spot of genomic gain found in many of these cancers. Depletion of kif14 in cultured human cancer cells lead to chromosome segregation defects, cytokinesis failure, and apoptosis. Despite its importance, kif14 is one of the most understudied kinesins. There are less than 40 articles on kif14 published to date. The molecular basis of kif14’s role in tumorigenesis and in mitosis remains largely unknown. We have successfully purified active recombinant kif14 constructs and solved the crystal structure of Kif14 motor domain (Arora et al., J Mol Biol. 2014). Our data revealed many properties of Kif14 that are distinctly different from conventional microtubule-based motors. The goal of the internship project is to understand how these characteristics link to kif14 functions and to validate our findings in living cells. Results obtained from this study will be crucial to understand kif14’s role in cell division and tumorigenesis.

LAB TECHNIQUES

Molecular biology
Protein biochemistry
High-resolution microscopy
Cell culture

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/benjamin-kwok
PROJECT DESCRIPTION

The formation of the mitotic spindle, a microtubule-based machine, is required for chromosome segregation during cell division. Inhibition of spindle assembly blocks cell division and is a viable mean to treat cancer. Paclitaxel, one of the most successful chemotherapeutics, targets tubulin, which is the building block of microtubules, and inhibits its polymerization dynamics. However, its success has been limited by the development of drug resistance in patients. Therefore, alternative strategies are needed to overcome this hurdle. Kinesin motor proteins which have the ability to control microtubule organization and polymerization dynamics provide attractive targets for chemical inhibition. Recently, we have completed two high-throughput screens to identify small molecule chemical inhibitors for kinesins. From 110,000 compounds that we have screened, we obtained about more than one hundred candidate hits with different level of selectivity against different kinesin families. We have now completed the initial phase of characterizing the candidate hit compounds in vitro using biochemical assays and in cells using high-resolution microscopy. In fact, we have published the characterization of one of these compounds recently (Talje et al., FEBS Lett. 2014). This internship project is to help determine the precise mechanisms of action of these kinesin inhibitors on enzymatic activity of the motor proteins and their impact on mitotic processes such as spindle assembly and chromosome segregation. Our ultimate goal is to understand how we can use these small molecules to suppress cell proliferation as a way to treat cancer.

LAB TECHNIQUES

- Biochemistry
- Cell culture
- Microscopy

FOR MORE INFO

www.irim.ca/en/research/principal-investigators/benjamin-kwok
PROJECT DESCRIPTION

How do adult stem cells divide in vivo, in response to niche signaling? Answering this question has been challenging, mainly due to the lack of appropriate models to visualize stem cells in vivo. We have developed a novel method to image the division of adult stem cells in vivo, using the nematode Caenorhabditis elegans (C. elegans) as model organism. Genetic analysis has revealed specific pathways and regulators that are essential to coordinate stem cell division during development and aging of the animal. We are seeking motivated individuals to pursue the characterization of some of these regulators, to better understand how they contribute to the regulation of stem cell division, using genetic analysis and high-resolution time-lapse imaging approaches.

LAB TECHNIQUES

RNAi
Genetic analysis
In vivo time-lapse imaging
Quantitative image analysis
Caenorhabditis elegans

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/jean-claude-labbe
PROJECT DESCRIPTION

The goal of this project is to develop a novel tree-based visualization tool that will allow researchers to efficiently compare and browse genomics data obtained on many patients.

Due to the advent of next-gen sequencing, more and more personalized medicine studies revolve around the study of polymorphisms or other specific and sparsely disseminated genomic data. Researchers often have to screen thousands of regions to be able derive meaningful conclusions from such data. Due to our lack of adequate visualization tools, this process can take days or weeks depending on the size of the dataset.

The ideal tool should be user-friendly and able to seamlessly cope with huge amounts of data. At IRIC we have developed pyGeno, a python package that allows users to query genomes in a fast and memory efficient way. The internship will consist in developing a client/server web application on top of pyGeno.

LAB TECHNIQUES

Bioinformatics
Proteogenomics
Computer programming in Python
RNA-Seq data analysis

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/sebastien-lemieux
Circos plots are now commonly seen in genomic related papers. They provide an easy way to present a number of different types of information related to chromosomal location. However, generating high-quality Circos plots can be time consuming. You need to think carefully about the message you want to share, then get all the data in the right format, create the configuration files for the perl application, see what works or not. Running Circos takes each time several minutes. Going through this error-trial process can be time-consuming.

An interactive tool to construct the Circos plot would greatly streamline this process and enable researchers at IRIC to better take advantage of this visualization tool. It would minimize the time spent in the error-trial period. The tool developed could generate the files needed to run the perl version when satisfied or run it for the user. Or it could provide a complete and interactive reimplementation of Circos.

**LAB TECHNIQUES**

- Bioinformatics
- Computer programming (Python, Javascript, HTML/CSS)
- Next-gen sequencing data analysis

**FOR MORE INFO**

PROJECT DESCRIPTION

In mammals, ATP-dependent SWI/SNF-like BAF (Brg/Brm-associated factor) chromatin remodeling complexes are composed of a dozen families of subunits assembled around two alternative ATPases, Brg and Brm. Increasing evidence indicates that combinatorial assembly of families of subunits confers functional specificity to these complexes by creating distinct polymorphic surfaces for interaction with specific histone codes, regulatory elements and/or DNA-binding factors. We recently showed that specialized BAF assemblies—with unique subunit composition—perform instructive and programmatic roles in embryonic stem cells (esBAF), neural progenitors (npBAF) and postmitotic neurons (nBAF). However, their role and mechanism of action in the developing hemopoietic system and in leukemia remain largely unexplored. Using proteomics and molecular genetics approaches, we obtained evidence that subunit exchange within a novel family of hemopoietic BAF complexes is essential for normal and leukemic hemopoiesis. In particular, we discovered a key component of the code as being the mutually exclusive usage of the BAF60a/b/c subunits. Using conditional (floxed) alleles, we showed that 60a and 60b are master regulators of lymphoid and granulocytic development, respectively, whereas preliminary studies suggest a role for BAF60c in regulating hemopoietic stem cell (HSC) function.

This research proposal aims at characterizing the molecular basis for lineage determination that emerges from combinatorial assembly of the BAF60a/b/c family of subunits within BAF complexes. More specifically, the trainee will work in collaboration with a Ph.D. student to determine the molecular and cellular mechanisms underlying BAF60a and 60b function in hemopoiesis.

LAB TECHNIQUES

Flow cytometry
Cell culture
Generation of retroviruses
Biochemistry
Molecular biology

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/julie-lessard
PROJECT DESCRIPTION

Progression through the cell cycle is controlled by cyclin-dependent kinase (Cdk) activity. Cdns are regulated positively by association with cyclins and negatively by binding to Cdk inhibitors. Among the Cdk inhibitors, p27 and p21 plays a major role in connecting mitogenic signaling pathways to the cell cycle machinery. The expression of p27 and p21 is high in non-cycling cells, and declines in response to mitogenic or oncogenic stimulation. The abundance of Cdk inhibitors is controlled by the ubiquitin-proteasome system (UPS). We have recently conducted loss-of-function RNAi screens of the human deubiquitinating enzyme (DUB) family to identify novel UPS regulators of p27 and p21. The objectives of this project are to investigate the biochemical basis of the regulation of Cdk inhibitors by DUBs and to evaluate the functional impact of these regulatory events on the cell cycle.

LAB TECHNIQUES

Cell biology
Molecular biology
Cell culture
Transfection
Immunohistochemistry
Cell proliferation assays
RNA interference
Affinity purification mass spectrometry

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/sylvain-meloche
PROJECT DESCRIPTION

The RSK family of protein kinases comprises four members (RSK1-4) that regulate cell proliferation, survival and motility in response to growth signals. These protein kinases are normally activated by the Ras/MAPK signaling pathway, which is frequently hyperactivated in human malignancies, including melanoma, lung and colorectal cancers. With the help of high-throughput DNA sequencing of human tumors, several studies have identified a large number of somatic mutations in the genes encoding RSK isoforms. The main goal of this project is to generate these mutations experimentally and to verify their impact on the catalytic activity of the different RSK isoforms. With the help of a doctoral student, the intern will be responsible of generating point mutations by site-directed mutagenesis and verifying the integrity of the mutated proteins. These proteins will then be transiently expressed in human cells and catalytic activity will be measured in vitro and in vivo. This project will help determine whether the identified somatic mutations in the RSK genes contribute to cancer progression.

LAB TECHNIQUES

Immunoblotting
Transfection
Cell culture
Mutagenesis
DNA cloning
Immunoprecipitation
Kinase assay

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/philippe-roux
www.roux.iric.ca
PROJECT DESCRIPTION

The aim of this project is to generate an experimental AML cell line from hematopoietic stem cells that are purified from human cord blood. The cell line will be generated to express both the MLL-AF9 leukemia oncogene and a doxycycline-responsive transcriptional activator. This will allow the doxycycline-inducible expression of shRNA constructs knocking-down essential regulators of AML progression and thereby study their molecular functions.

LAB TECHNIQUES

Purification and culture of human hematopoietic stem cells obtained from cord blood
Genetic manipulation of cells by transfection and viral transduction
Biochemical and molecular biology methods (PCR, Southern blot, Western analysis)
Functional validation in tissue-culture and in vivo based experiments

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/guy-sauvageau
PROJECT DESCRIPTION

The Ras/MAPK pathway plays a pivotal role in the control of cell proliferation and its aberrant up-regulation often leads to cancer. Signaling through the Ras/MAPK pathway depends on a number of core pathway components that include a module a three kinases known as RAF, MEK and MAPK. We have recently completed a genome-wide functional (RNAi-based) screen to identify additional factors modulating Ras/MAPK signaling. Intriguingly, while conventional signaling components were identified, we also observed that several hits played a role in controlling the steady-state levels of MAPK. In particular, we identified factors of the Ubiquitin/Proteasome System (involved in protein degradation) that control the half-life of MAPK. A position for a summer student is available in the laboratory for initiating the characterization of the mechanism of action of some of these new factors.

LAB TECHNIQUES

Plasmid constructs
DNA sequence analysis
CRISPR-based gene knockouts
Cell culture
Cell transfection
Protein immunoprecipitation
Protein separation on SDS-PAGE gels
Western blotting

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/marc-therrien
PROJECT DESCRIPTION

Our laboratory discovered a novel and fascinating feature of chromosome physiology. During DNA replication, chromatin structure needs to be duplicated and this is achieved through the incorporation of newly synthesized histones into nascent chromatin. We showed that new histone H3 molecules deposited throughout the genome are acetylated at lysine 56 (H3K56ac). Both H3K56ac and deacetylation play important roles in the response to a broad spectrum of DNA damaging agents that are frequently used in cancer chemotherapy (Nature (2005) 436: 294; Curr Biol (2006) 16: 1280; Cell (2008) 134: 244; Mol. Cell. Biol. (2012) 32: 154).

Notably, we found that the enzyme that deacetylates H3K56, known as Hst3, is conserved in many human pathogens such as 

\textit{Candida} and \textit{Aspergillus} species. Remarkably, inhibition of Hst3 using nicotinamide, a form of vitamin B3, leads to catastrophic chromosome damage and fungal cell death (Nature Medicine (2010) 16: 775). Thus, inhibitors of Hst3 represent a novel therapeutic approach to treat fungal infections that can be life-threatening in patients that are immunosuppressed (e.g. patients undergoing cancer chemotherapy or organ transplant). For these reasons, we are actively studying the mechanism of action and the regulation of the deacetylase Hst3.

The aim of this project will be to decipher the mechanisms that regulate the activity and the stability of the Hst3 enzyme during the cell cycle and in response to DNA damaging agents.

LAB TECHNIQUES

- Cell synchronization
- Immunoblotting
- DNA damage sensitivity assays
- Immunoaffinity purification

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/alain-verreault
PROJECT DESCRIPTION

The focus of our lab is in understanding the biology of leukemias using various high-throughput approaches. In order to do this we compare the RNA/DNA of human leukemias that we have sequenced and a unique human model leukemia system that allows us to generate human leukemias from healthy cord blood cells. Our research has now highlighted a small group of 30 genes that are specifically expressed in leukemias, but not normal cells (e.g. they are biomarkers for leukemia. Because the genes are consistently expressed in leukemias, it implies that at least some of these are important for the growth/development of the leukemia. The goal of trainees who come to the lab is to help in discovering what the functions of these genes are. We assess this in a variety of ways including “knocking down” the expression of genes using shRNAs and assessing growth, treating with specific chemical inhibitors, overexpressing genes which are known to regulate the target genes, etc. The candidate will participate in the design of the experiments, as well as the lab work and analysis of the results. Several candidate genes are currently being examined and the candidate will work under the supervision of a post-doctoral fellow in the lab. Aspects of the project (e.g. bioinformatics analysis) can be tailored to suit the background of the successful candidate.

LAB TECHNIQUES

Next generation DNA/RNA sequencing
Western blotting
RT-PCR/qPCR
Cell culture
shRNA KD experiments
Flow cytometry
Bioinformatics

FOR MORE INFO

www.iric.ca/en/research/principal-investigators/brian-wilhelm